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STUDIES ON THE EXCRETION AND DISTRIBUTION OF RADIOACTIVE NICOTINE AND SOME EVIDENCE ON THE URINARY METABOLITES(1)

BY

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Radioactive nicotine was first used by GANZ and KELSEY (1) and by GANZ, KELSEY and GEILING (3) to study the urinary excretion in the rat and mouse. The radioassay techniques of KELSEY (2) were used and it was shown that nearly all of the radioactive drug was found in the urine. TEDESCHI *et al.*, (4) and BENNETT *et al.*, (5) used radioactive nicotine to give an 8 hour I. V. infusion to dogs and OWEN, *et al.*, (6) using cats again found nearly all of the radioactivity in the urine. This paper confirms the urinary excretion in the rat and dog and presents data for the guinea pig. Additional data on tissue distribution in the rat, a single tissue distribution in a dog, and radioautographic chromatograms of dog urine are presented. At the time these experiments were done the number of observations was limited by the small supply of radioactive nicotine which was then available.

METHODS AND RESULTS

Radioactive nicotine was prepared by growing N. RUSTICA in an atmosphere of $C^{14}O_2$ by the method of GANZ, *et al.*, (3) and samples were counted for a period of time so that the standard error was 5 % or less. Eight Sprague Dawley rats weighing from 200 to 400 gms were used to study the tissue distribution of carrier-free radioactive nicotine.

(1) This study was performed partly at the Department of Pharmacology, University of Chicago; St. John's Hospital, Lowell, Massachusetts; and Presbyterian Medical Center, San Francisco, California.

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TABLE I

Three hour Distribution of Radioactive Nicotine in the Rat C^{14} Calculated as Nicotine

% = Percent of Total Radioactivity Administered

	Kidney		Brain		Lung		Liver		Skeletal Muscle	Spleen		Heart	
	μgms/gm	%	μgms/gm	%	μgms/gm	%	μgms/gm	%	μgms/gm	μgms/gm	%	μgms/gm	%
Rat 1	2.8	1.4	1.1	.53	3.2	1.2	2.6	6.6	—	—	—	—	.37
2	2.5	1.34	.62	.29	1.21	.44	1.2	2.82	—	—	—	—	—
3	2.03	1.17	.67	.29	2.62	1.25	1.39	3.36	—	—	—	—	.25
4	2.26	1.24	1.0	.29	2.98	1.09	1.87	3.73	.92	—	—	—	.85
5	2.44	1.24	1.13	.52	.85	.31	.41	.86	1.0	—	—	—	.25
6	1.61	.88	.73	.35	1.33	.69	1.17	2.9	.87	1.14	.66	.81	.23
7	1.63	.9	.66	.31	1.05	.38	1.5	3.17	.83	.93	.44	.83	.19
8	1.46	.58	1.62	.57	1.6	.46	1.5	2.42	.95	.51	.33	.88	.17
AVE.	2.09	1.09	0.94	.39	1.86	0.73	1.46	3.23	.91	.86	.48	.84	.33

A dose of 1.4 mg/kg was given I. P. and the animals were sacrificed after 3 hours. The data tabulated in Table I shows that the kidney contains the highest concentration of radioactive compounds on a per gram basis followed by the lung, liver, brain, skeletal muscle, spleen and heart. Since the main excretory pathway is via the urine, it is expected that the kidney would show higher concentrations of radioactivity. The exhaled CO_2 was collected in NaOH and precipitated as $BaCO_3$. No activity was found in the exhaled CO_2.

TABLE II

Eighteen Hour Excretion of C^{14} Labeled Nicotine in the Guinea Pig
(Percent of Total Radioactivity Administered)

	Urine	Feces
G.P. 1	100	—
2	92.6	—
3	73	—
4	100	—
5	100	0.4
6	87.5	4.05
7	89.2	1.89
AVE	91.7	2.11

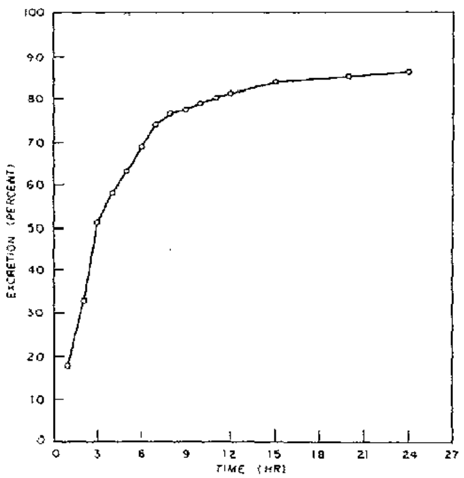

FIG. 1

24 Hour urinary excretion of radioactive nicotine in dog.

Seven guinea pigs ranging in weight from 400 to 700 gms. were given 5 mg/kg I. P. injection of radioactive nicotine. The urine and feces were

collected at 18 hours. Table II shows that the main excretory pathway in the guinea pig is the urine and is about 90 % complete in 18 hours. This is in agreement with the data from the rat, cat, mouse and dog. The feces accounts for very small amounts of drug, being about 2 % of the total dose. The exhaled CO_2 was examined for $C^{14}O_2$ and none was found.

Four dogs were anesthetized with nembutal and given 1 mg/kg I. V. dose of radioactive nicotine. One dog was given a dose of 4 mg/kg I. V. The urine and feces were collected each hour and assayed. Dog No. 2 was sacrificed at 3 hours for a tissue distribution study. The urine of the dog has been shown to be the major excretory pathway (4,5) and that data in Table III confirms this conclusion. (See FIG. 1). In our dog experiments more than 75 % of the total dose was found in the urine by 24 hours while the feces (TABLE IV) contained about 2 % of the total dose, in one dog there was 7 % in the feces at 24 hours. By 48 hours nearly all of the administered activity had been recovered. The higher dose (4 mg/kg) did not change the rate of excretion in the single observation which we made.

TABLE III

% Cumulative Urinary Excretion of Total Activity in the Dog

	Dose Iv.	HOURS						
		1	2	3	16	19	24	32
Dog 1 ♀	1 mg/kg	18.7	34.0	44.7			77.0	
Dog 2 ♂	4 mg/kg	—	—	47.0		71.9		90.8
	1 mg/kg	4.33	36.43	43.27		sacrificed		
Dog 3 ♀	1 mg/kg	14.69	25.5	32.13	53.8			
	1 mg/kg	13.5	37.8	56.3	91.7		94.6	
Dog 4 ♀	1 mg/kg	17.1	32.8	51.2	83.71	84.98	86.38	

A single tissue distribution was performed on dog No. 2 three hours after the I. V. administration of 1 mg/kg radioactive nicotine. The dog was anesthetized with nembutal. Table V shows the organs listed in order of highest affinity for accumulating the nicotine and degradation products. The stomach wall had a greater affinity for the drugs than did

TABLE IV

% Cumulative Feces Excretion of Total Activity in the Dog

	Dose Iv.	3 Hr.	24 Hr.	40 Hr.	48 Hr.
Dog 1	1 mg/kg		2.54		
Dog 2	Sacrificed at 3 hrs.				
Dog 3	1 mg/kg		7.23		
	1 mg/kg				2.17
Dog 4	1 mg/kg	1.09		1.61	

TABLE V

Three Hour Tissue Distribution of Radioactive Nicotine in the Dog

Dog 2. 1 mg/kg Iv. Nembutal anesthesia

Organ	% of total dose	μgms/gm
Stomach	32.5	29.6
Liver	20.9	4.95
Stomach contents	3.68	—
Small intestine	3.14	0.9
Lung	1.92	1.42
Kidney	1.08	1.65
Heart	0.83	1.08
Small intestine contents	0.59	—
Large intestine	0.49	0.86
Bile	0.365	—
Pancreas	0.22	0.74
Spleen	0.175	0.86
Brain	0.173	0.22
Large intestine contents	0.133	—
Urinary bladder	0.128	1.3
Gall bladder	0.102	2.07
Adrenal	0.02	1.08
Saliva	—	0.05 μgms/cc
Mesentery fat	—	0.52
Subcutaneous fat	—	insignificant
Perirenal fat	—	insignificant
Skin	—	insignificant
Urine	43.2	

the liver, the bile had only small quantities and the brain is found even further down in the list of decreasing activities. Saliva, fat and skin did not contain measurable amounts of radioactivity.

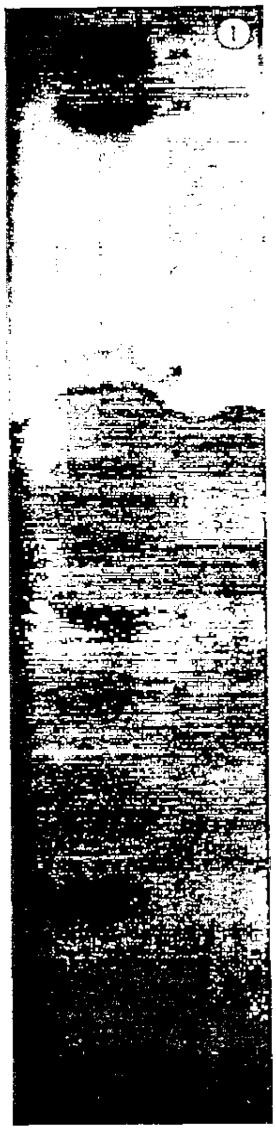

FIG. 2

Dog urine (descending column) butanol saturated with 1 % HCl 415 days exposure.

1. Excreted Urine.

FIG. 3

Dog urine (descending column) *n*-butanol saturated with 1 % HCl 413 days exposure.

1. Excreted Urine.
2. Boiled Urine.
3. Nicotine Standard.

The degradation products of nicotine have been studied by others and little is known regarding the number of metabolites or their structure. In order to try to establish some preliminary information on the number of metabolites we collected a 24 hour sample of urine from Dog 2 after I. V. administration of 4 mg/kg carrier-free radioactive nicotine. The pooled urine was lyophilized and a concentrated water solution was made. Because of the quantity of solids it was possible to plate only 2000 counts

FIG. 4

Dog urine (descending column) butanol saturated with 1 % HCl 580 days exposure
1. Toluene Extract
2. Benzene "
3. CCl_4 "
4. $CHCl_3$ "

Dog urine (descending column) butanol saturated with 1 % HCl 579 days exposure.
5. Acetone Extract
6. Excreted Urine
7. Urine and Nicotine
8. Ethyl Acetate

on a strip of Whatman 1 filter paper. To obtain information on the solubility of the break-down products, aliquots of 2000 counts was exhaustively extracted with toluene. The toluene was then evaporated to dryness in air and the residue re-dissolved in toluene. This was then

FIG. 5

Dog urine (descending column) *n*-butanol saturated with 1 % NH_4OH 578 days exposure.

1. Toluene Extract
2. Benzene "
3. CCl_4 "
4. $CHCl_3$ "

plated on another strip of filter paper. This same procedure was followed using benzene, carbon tetrachloride, chloroform, acetone and ethyl acetate. As a control a paper strip was spotted with a mixture of lyophilized urine and radioactive nicotine. Each paper strip was chromatographed in a descending column using a system of butanol saturated with 1 % HCl and also in butanol saturated with 1 % NH_4OH. An aliquot of excreted urine (not lyophilized), an aliquot of boiled urine, and a nicotine standard was run in butanol saturated with 1 % HCl. After the chromatograms had traversed the length of the paper strip (2 ran off) they were air dried and placed in non-screen X-ray film holders with non-screen X-ray film and allowed to expose for a period of time from 413 to 580 days. The film was developed in complete darkness in X-ray developer.

The spots of activity on the film represent the separation of the degradation products or only those which were soluble in the different solvents. Each spot is some fraction of 2000 counts and the various densities gives an indication of the relative quantities present. No attempt was made to further isolate or characterize these products. Figure 2 shows 10 separate spots from the unlyophilized pooled urine. Figure 3 is a composite of three samples which were run on large sheets of filter paper. The excreted urine (FIG. 3, No. 1) did not show more than three well defined areas of activity; Fig. 3, No. 2 is boiled urine and this was more easily read with nine active areas showing; Fig. 3, No. 3 is the nicotine standard and one major spot besides two smaller spots are found. We do not know what the areas of low activity are and they were not suspected until they appeared on the films. They apparently are in very minute amounts and were carried through a rigorous separation and purification procedure. Figure 3 shows the solubility of the metabolites in six solvents. Toluene (FIG. 4, No. 1) carries three spots, benzene (FIG. 4, No. 2) carries two spots, carbon tetrachloride (FIG. 4, No. 3) carries two spots, chloroform (FIG. 4, No. 4) carries five spots, acetone (FIG. 4, No. 5) carries one spot, Nos. 6 and 7 ran off, ethyl acetate (FIG. 4, No. 8) carries five spots. Using a system of butanol saturated with 1 % NH_4OH readable films were obtained only with toluene (FIG. V, No. 1) which carries two spots, benzene (FIG. 5, No. 2) which carries two spots, carbon tetrachloride (FIG. 5, No. 3) which carries two spots and chloroform (FIG. 5, No. 4) which carries four spots.

DISCUSSION

The three hour tissue distribution in the mouse (3) showed that nicotine and metabolites accumulate by weight in order of decreasing

TABLE VI

Rf Values of Dog Urine Chromatograms

FIG. 2

.028
.056
.108
.36
.47
.58
.66
.73
.77
.85

FIG. 3

No. 1	No. 2	No. 3
.02	.027	.13
.08	.08	.44
.84	.43	.62
	.55	
	.63	
	.73	
	.77	
	.83	
	.96	

FIG. 4

No. 1	No. 2	No. 3	No. 4	No. 5	No. 8
.69	.85	.84	.43	.85	.60
.90	.93	.92	.70		.65
.97			.75		.78
			.82		.82
			.90		.88

FIG. 5

No. 1	No. 2	No. 3	No. 4
.88	.93	.85	.77
.94	.97	.92	.81
			.86
			.92

concentrations in the kidney, liver, lung, heart, spleen, brain and skeletal muscle. In the rat our data shows the following pattern of deposition; kidney, lung, liver, brain, skeletal muscle, spleen and heart. The kidney is the actively excreting organ and has the highest conc/gm in both species. The exhaled breath of the mouse is reported to contain no $C^{14}O_2$ (3) and also none in the dog (5). Our study in the rat and guinea pig likewise showed no radioactivity in the expired air.

The rapid urinary excretion of nicotine has been reported in the rat (3) dog (4,5) and cat (6). Our data shows that urinary excretion in the guinea pig is also rapid and nearly complete in 18 hours. Fecal excretion amounts to a very small percentage of the administered dosage. The results on the urinary excretion of nicotine in four dogs agrees with the work of TEDESCHI, *et al.*, (4) and BENNETT, *et al.*, (5). The rapid and nearly complete recovery of radioactivity indicates that the metabolic degradation of the nicotine molecule proceeds immediately upon administration of the drug. The kidney is able to excrete both unchanged nicotine and the degradation products but when large doses are given the proportion of unchanged nicotine is greater (5) and varies with the pH of the urine (7). The radioactivity is distributed to nearly all organs of the dog. Notable are low concentrations in the brain which suggest that nicotine convulsions of CNS origin (8) may result from a very acute sensitivity probably to undetoxified nicotine and less sensitivity to the metabolites. The transient nature of the nicotine convulsion may thus be related to rapid destruction of nicotine in the liver (9). There appears to be no accumulated storage depot in the body and the body fat is not affected. The physiological degradation products of nicotine are not known. Attempts to characterize them by a color reaction with cyanogen bromide led CASTLE and BURGER (10) to the conclusion that B-amino-B-(3 pyridyl) propionic acid and B-methyl-amino-B-(3 pyridyl) propionic acid and possibly γ keto- γ (3 pyridyl) butyric acid are formed. MCKENNIS, *et al.*, (11,12) concluded that γ (3 pyridyl)-γ-methylamino butyric acid is found in dog urine. OWEN and LARSON (6) made paper chromatographs of radioactive urine from dogs and determined the areas of activity by cutting the paper into 2.2 cm. squares and measuring in a gas flow counter. This method is not accurate but has the advantage of being rapid. They found the presence of three major metabolites and four minor metabolites. Our chromatograms were exposed to film for about one and a half years. We are able to determine ten separate areas of exposure and conclude that there may still be more than one metabolite in each separation, but the evidence for at least ten metabolites is convincingly strong. When the urine was boiled and chromatographed

we find the presence of at least nine metabolites. In each of these paper strips, unchanged nicotine is assumed to be present. The metabolites are found to be more soluble in chloroform and ethyl acetate; less soluble in toluene, carbon tetrachloride, benzene and acetone. It would not appear unreasonable to be suspicious of more than ten metabolites. In studies on the bacteriological degradation of nicotine, FRANKENBURG and VAITEKUNAS (13) determined that there is a possibility of seventeen metabolic derivatives culminating in methylamine, ammonia and oxalic acid.

Other methods of preparing radioactive nicotine have been shown to provide specific labelling in the molecule. Feeding certain amino acids to tobacco plants results in integration into the molecule. Using radioactive ornithine plant feeding, a labelled nicotine was prepared by LEETE (14). DEWEY, *et al.*, (15) determined that the incorporated ornithine is largely in the 3, 4 and 5 positions of the pyrrolidine ring. He suggests that probably 50 % may be in the 5 position and less than 2 % in the N-methyl group. BYERRUM, *et al.*, (16) showed that tobacco plants will take up serine and formaldehyde. The latter was found to account for 90 % of the activity of the nicotine molecule in the N-methyl group. Methionine and glycine also are introduced into the N-methyl position. After feeding tryptophane (17) no activity was found. Glutamic acid was incorporated into the 2 and 5 position of the pyrrolidine ring in about equal amounts (18). Nicotinic acid is incorporated into the nicotine molecule in the pyridine ring and the suggestion by DAWSEN, *et al.*, (19) is that nicotine may be a detoxification end product in plant metabolism. KUZIN and MERENOVA (20) observed that nicotine distilled from isolated tobacco leaves had weak radioactivity. They prepared nicotine picrate and oxidized it with nitric acid to nicotinic acid. The nicotine picrate had shown activity but there was none in the nicotinic acid, thus no labelling occurred in the pyridine ring. Further experiments using nicotine oxidation by selenium dioxide confirmed the presence of radioactivity in the methyl group of the pyrrolidine ring. If nicotine from a tobacco plant is similarly labelled as is the isolated leaf, we can conclude that the ten metabolites (FIG. 2) are degradation products involving derivatives of the N-methyl group of the pyrrolidine ring.

SUMMARY

1. Distribution of radioactive nicotine in the rat shows the most active concentrating organs to be kidney, lung and liver.

2. The guinea pig excretes 90 % of the administered nicotine in 18 hours via the urine.

3. The dog excretes all of the nicotine within 48 hours, only 2-7 % being found in the feces.

4. No radioactivity was found in the expired air of the rat or guinea pig.

5. Distribution of nicotine in the dog (in a single observation) shows highest activities in the stomach and liver. No activity in saliva, skin and adipose tissue.

6. A radioautograph of a dog urine chromatogram shows at least ten compounds. Solubility data is presented.

7. Two additional radioactive substances were found in a control chromatogram of the radioactive nicotine. They occurred in very small quantity and their identity has not been determined.

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